

BIOLOGICAL AND MORPHOLOGICAL ASPECTS OF THE EGG
STAGE AND OVIPOSITION OF *LANGURIA MOZARDI*
(COLEOPTERA: LANGURIIDAE)

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Abstract.—Oviposition sites of *Languria mozardi* Latreille, the clover stem borer, were determined on *Trifolium incarnatum* L., a previously unreported host. The 2nd and 3rd internodes of primary flowering stems are the preferred sites on *T. incarnatum*. Oviposition occurs in stems of *T. repens* L., *T. pratense* L., *T. hybridum* L., *T. resupinatum* L., *T. striatum* L., *T. nigrescens* Viv., and *T. subterraneum* L. but not on *T. lappaceum* L.. There is usually one oviposition puncture and egg per stem, the egg being attached to the plant tissue by an extension on the posterior end. The micropylar region is simple in form and the chorionic surface has a granular appearance. The sensilla on the ovipositor differ in the types, number, and distribution as compared to the sensilla on the ovipositor of other beetle species. The ablation experiment indicates that these sensilla on the ovipositor are possibly involved in selecting a suitable oviposition site.

Key Words: *Languria mozardi*, sensilla, larva, mouth parts, antenna

The clover stem borer, *Languria mozardi* Latreille, is endemic to North America on a wide range of host plants including the Leguminosae, Compositae, Umbelliferae, and Gramineae. It is considered a pest of alfalfa, *Medicago sativa* L., and red clover, *Trifolium pratense* L. (Lintner 1881, Folsom 1909). Wildermuth and Gates (1920) reported early studies on the biology, morphology, and pest status of *L. mozardi* on forage crops. Damage from larval feeding inside stems weakens the plant and results in lodging, loss of seed, and forage production. Damage to stems also may predispose plants to root rot pathogens. Increased fiber content in damaged stems reduces forage quality (Wildermuth and Gates 1920).

Except for a single report (Knight et al. 1976) from arrowleaf clover, *T. vesiculosum*

Savi, *L. mozardi* is known as a forage pest only on perennial legumes. This insect has been collected annually in Mississippi from 1982-85 on *T. vesiculosum*, *T. incarnatum* L. (crimson clover) and from 1983-85 on *T. alexandrinum* L. (berseem clover). The importance of seed production in annual clovers to ensure stand persistence and the ability of *L. mozardi* to reduce seed and forage yield in other legumes makes this insect a potential pest of annual clovers.

No information is available on the biology of *L. mozardi* on annual clovers and existing morphological studies are incomplete. In the present study, oviposition sites and frequencies on crimson clover, a previously unreported host, are reported from greenhouse studies. Host range for oviposition also are determined on several clover

species that are or have been of economic importance. The morphology of the egg and ovipositor sensilla are described.

MATERIALS AND METHODS

Adult *L. mozdari* were collected from roadside plantings of crimson clover using a motorcycle-mounted collection net (Ellsbury and Davis 1982). Pairs of mating *L. mozdari* were sorted from collections, provided with a water source, and held 24 h in a 9-cm diam plastic Petri dish. To determine oviposition sites on crimson clover, 3 pairs of insects were confined for 24 h on each of 12 greenhouse-grown flowering crimson clover plants in cylindrical screened cages (14-cm diam \times 90 cm). Host range for oviposition by *L. mozdari* on other *Trifolium* species also was studied by similarly caging 3 pairs of insects on each of 2 greenhouse-grown flowering plants of *T. repens* L. (white clover), *T. pratense* (red clover), *T. hybridum* L. (alsike clover), *T. resupinatum* L. (persian clover), *T. striatum* L. (pitts or striate clover), *T. nigrescens* Viv. (ball clover), *T. lappaceum* L. (lappa clover), and *T. subterraneum* L. (subterranean clover). After 24 h exposure to test insects, stems were split using a single-edge razor blade and the location and number of eggs were recorded for oviposition punctures on primary and lateral stems.

An average internodal location of oviposition punctures was calculated for clovers in the host range study from integer values assigned to each internode beginning with the lowermost internode (#1) and increasing to the (last) internode below the flower head of each stem.

Eggs were removed from the clover stems and placed in 5% glutaraldehyde in 0.1 M Na-cacodylate buffer at pH 7.2 for 12 h at 4°C. The specimens were washed in the same buffer and then post-fixed in 4% OsO₄ in cacodylate buffer for 8 h. After dehydration the eggs were critical-point dried, affixed to aluminum stubs with tape, sputter coated with Au/Pd, and examined with a Hitachi

HHS-2R scanning electron microscope at 20 kV. The same preparation procedure was used for the ovipositors. Measurements were made with a light microscope with a calibrated ocular micrometer and are given as a mean plus the range.

Mated females for the behavioral experiments were anesthetized with CO₂ and the ovipositor was gently pulled out and treated with 16% HCl. They were placed in petri dishes with a moist filter paper and allowed to recover overnight. Six treated and 6 untreated females were placed in petri dishes containing pieces of flowering clover stems with the second and third internodes. The specimens were examined every 10 minutes for 1 h and the experiment was replicated 3 times.

RESULTS

Frequency distribution by internode of oviposition sites on crimson clover containing 1 or 2 eggs is illustrated in Fig. 1. Oviposition punctures were most frequent (22/63 and 18/63, respectively) in the 2nd and 3rd internodes of primary flowering stems. No eggs were deposited in lateral flowering stems. Of 78 stems examined, 11 had 2 oviposition punctures and 41 had one puncture. Six of 8 sites where 2 eggs were deposited were situated at the 3rd internode. Mean number of eggs per stem was 0.86 ± 0.11 ($\bar{x} \pm SE$, $N = 78$).

Oviposition by *L. mozdari* in 11 species of clovers during a no-choice test is summarized in Table 1. Lappa clover, *T. lappaceum*, was the only species in which eggs were not deposited. The usual number of eggs per site is 1 or 2 for all clovers studied, although 3 eggs occurred in a single puncture on *T. nigrescens* and 4 eggs in a puncture on a stem of *T. pratense*. Eggs were usually deposited in sites at the 3rd internode or higher on all clovers studied. The greatest number of eggs per stem (0.75 ± 0.33) were deposited in red clover, *T. pratense*.

Behavior of the adult female during ovi-

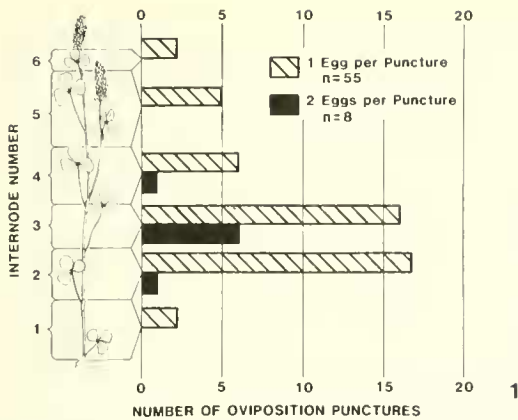


Fig. 1. Internodal frequency distribution of *Languria mozaridi* egg punctures containing 1 or 2 eggs in stems of crimson clover, *Trifolium incarnatum*.

position is similar on all *Trifolium* species included in these studies. After chewing a hole in the plant tissue the female uses her ovipositor to excavate a cavity in the pith into which the egg is placed. Externally, the opening is covered by shredded plant material while internally the opening is surrounded by a distinct rim (Fig. 2a, b). The egg is attached to the plant tissue by an extension from the tapered end (Fig. 2c). The egg is cylindrical, translucent yellowish, tapered at one end and measures 1545 μ m (1455–1800) in length and 495 μ m (480–

510) in width (Fig. 2d). The micropyle is situated on the blunt end and consists of a small, irregular shaped area from which several ridges radiate (Fig. 2e). At low magnification the surface of the chorion appears smooth (Fig. 2d) but at higher magnifications the surface has a rough, granular appearance and minute openings are dispersed randomly over the surface (Fig. 2f).

The ovipositor coxites are pointed distally and near the middle of the ventral surface of each coxite there is an area with a high concentration of long, slender spines (Fig. 3a, b). Just posterior to the distal end of each coxite a short style is present (Fig. 3a, b). It is 72 μ m (69–74 μ m) long and 20 μ m (18–21.5 μ m) wide and 10 sensilla trichodea are on the apex (Fig. 3b, d). These sensilla vary in length from 23.5 μ m (21–25 μ m) to 98 μ m (95–101 μ m) and they are either slightly curved or straight (Fig. 3d). Another group of 9 sensilla trichodea are situated on the ectal surface of each coxite near the base of the style (Fig. 3a, c). These trichoid sensilla range in length from 19 μ m (17.5–21 μ m) to 71.5 μ m (69–74 μ m). The 6 sensilla positioned between the straight single posterior and 2 long straight anterior sensilla are slightly curved (Fig. 3c). Sensilla basiconica are situated on the dorsal and

Table 1. Frequency and internodal sites of oviposition by *Languria mozaridi* in eleven *Trifolium* species during a no-choice test.

<i>Trifolium</i> Species ¹	No. Stems	Eggs/Stem ²	Mean No. Nodes/Stem	Ave. Internodal Location of Oviposition Punctures
* <i>T. subterraneum</i>	19	.47 \pm .16	6.42 \pm .55	4.67 \pm .69
<i>T. lappaceum</i>	22	0	9.14 \pm .60	—
* <i>T. nigrescens</i> ³	5	—	7.00 \pm .32	—
* <i>T. resupinatum</i>	26	.23 \pm .08	5.65 \pm .23	4.17 \pm .65
* <i>T. repens</i>	13	.38 \pm .14	7.96 \pm 1.04	3.40 \pm 1.17
* <i>T. striatum</i>	16	.44 \pm .18	6.25 \pm .40	3.00 \pm .82
<i>T. pratense</i>	12	.75 \pm .33	5.25 \pm .37	5.00 \pm .50
<i>T. vesiculosum</i>	16	.69 \pm .24	10.50 \pm .61	8.27 \pm .75
* <i>T. alexandrinum</i>	13	.23 \pm .12	12.85 \pm .69	7.67 \pm 1.45
* <i>T. hybridum</i>	9	.22 \pm .18	8.22 \pm .78	4.00 \pm 1.00
* <i>T. incarnatum</i>	16	.69 \pm 1.8	6.50 \pm .33	4.22 \pm 0.43

¹ *Trifolium* species preceded by * are previously unreported hosts.

² All numbers are mean \pm standard error.

³ Only one *T. nigrescens* plant used; 3 eggs laid in a single stem.

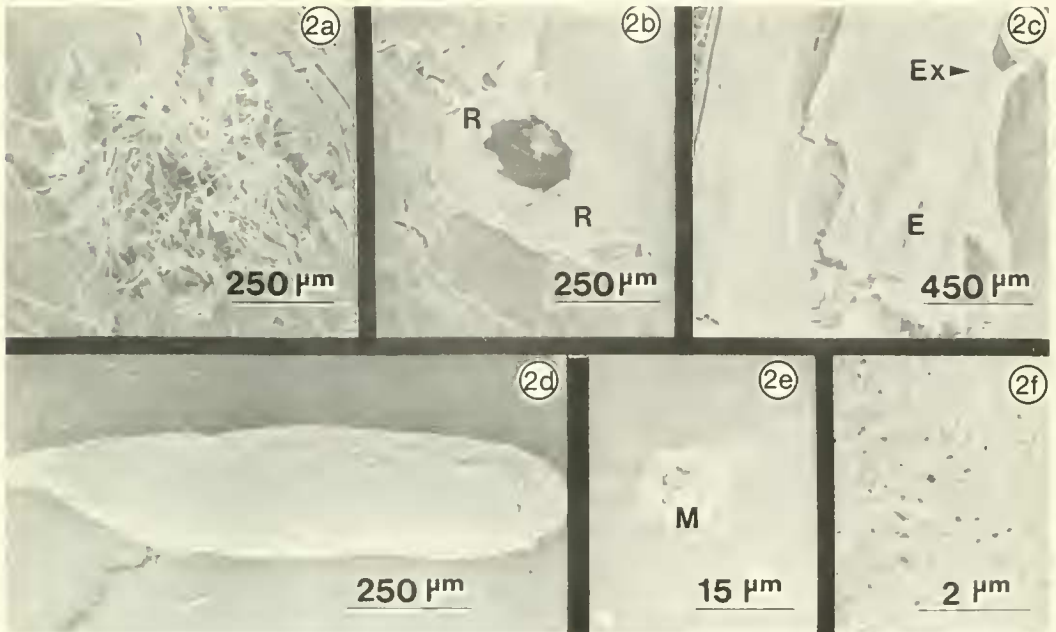


Fig. 2a-f. Egg of *L. mozdardi*. a, External appearance of the oviposition site. b, Rim surrounding the oviposition opening internally. c, Tapered end of the egg attached to the plant tissue. d, *L. mozdardi* egg. e, Micropylar area. f, Chorion surface. E = egg; Ex = egg extension; M = micropyle; R = rim.

ventral surfaces of the coxites (Fig. 3e). These basiconic sensilla are $5.5\ \mu\text{m}$ ($4\text{--}6\ \mu\text{m}$) in length and are situated on a cuticular depression (Fig. 3e). There are pores (ca. $1\ \mu\text{m}$ wide) on both surfaces which are interspersed among the basiconic sensilla (Fig. 3e). The sensilla trichodea and basiconica are stained with the silver nitrate.

In the behavioral experiments, none of the 18 treated females excavated any oviposition sites on the stems or laid any eggs whereas the untreated females made oviposition excavations and deposited eggs. Many of the treated females left the stems after 20 to 30 minutes.

DISCUSSION

Oviposition behavior of *L. mozdardi* in crimson clover is consistent with that reported by Folsom (1909) in red clover and by Wildermuth and Gates (1920) in alfalfa. Eggs are usually laid singly but occasionally 2 or more are deposited in a single puncture. Normally one, and infrequently 2, puncture(s) are found per stem.

The distribution of oviposition sites may be an important consideration in sampling for clover stem borer egg populations in crimson clover. Most oviposition (54/63 sites) occurs in the stem interval between the 2nd and 4th internodes. Sampling time for egg-infested crimson clover stems could be reduced by limiting samples to stem sections including only the 2nd through 4th internodes.

Previously reported hosts of *L. mozdardi* in the genus *Trifolium* include only *T. pratense* (Lintner 1881, Folsom 1909) and *T. vesiculosum* (Knight et al. 1976). Since oviposition by *L. mozdardi* occurs in 7 additional *Trifolium* species (Table 1) these clovers also should be considered potential hosts for this insect pending confirmation of their suitability as hosts for larval development.

The excavation for oviposition is similar to that described for *L. mozdardi* by Girault (1907) but he does not mention the rim surrounding the opening internally. The egg shape of *L. mozdardi* differs from *Languria*

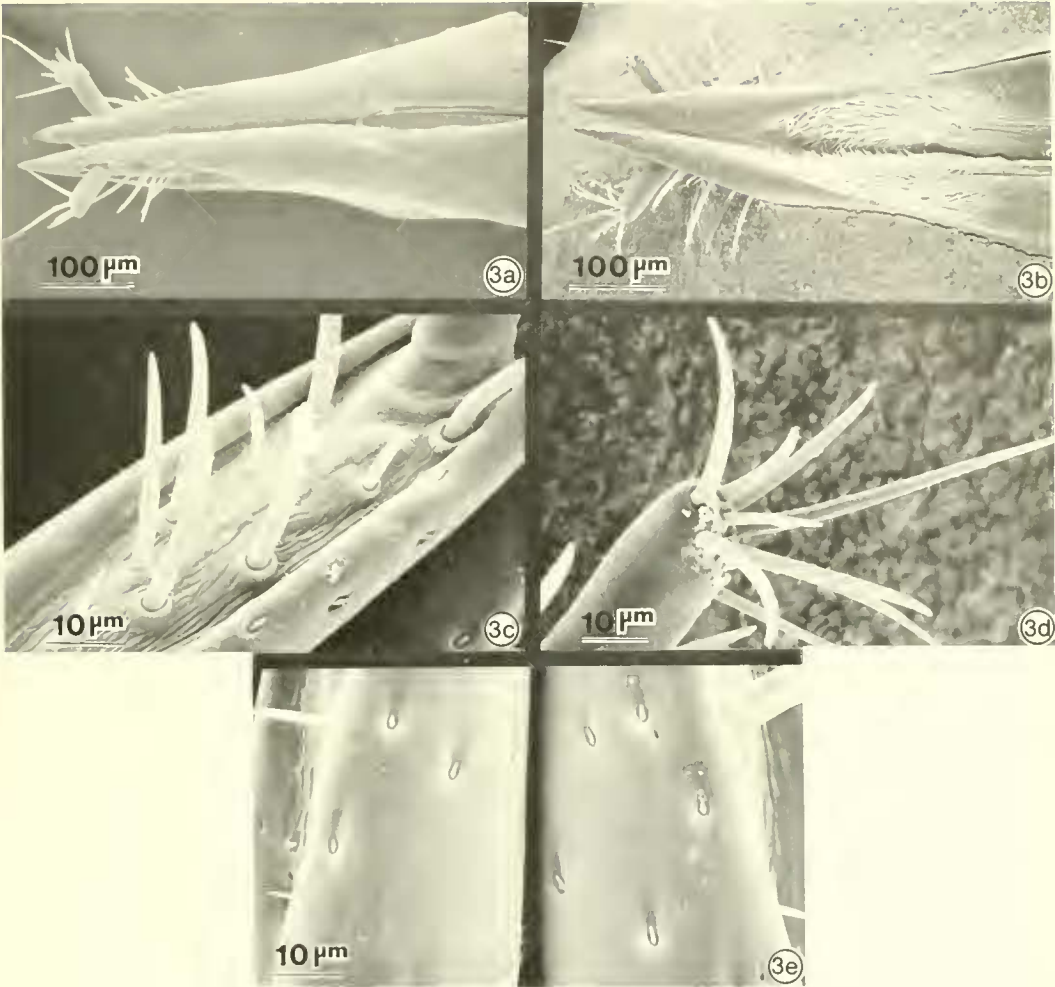


Fig. 3a–e. Ovipositor of *L. mozardi*. a, Dorsal surface. b, Ventral surface. c, Sensilla at the base of the stylus. d, Sensilla on the apex of the stylus. e, Short sensilla basiconica on the dorsal surface.

erythrocephalus Blatchley and *Acropteroxys gracilis* (Crotch) in that the end opposite the micropyle is more tapered in *L. mozardi* as compared to a blunt end in the other 2 languriid species (Piper 1978, Genung et al. 1980). The extension from the tapered end of the egg is used to attach the egg to the plant tissue and this structure was not observed in other languriid species but is similar in structure and function to the one found on the egg of *Lyctus brunneus* (Stephens) (Iwata and Nishimoto 1982). Sensilla are situated on the apex of the styli and near the base of the styli in other

beetles such as *Acanthoscelides obtectus* Say (Bruchidae) (Szentesi 1976), *Altica lythri* Aube (Chrysomelidae) (Phillips 1978), and *Thanasimus dubius* (F.) (Cleridae) (Baker and Nebeker 1986). There are differences in the types and numbers of sensilla on the ovipositor of the three previously mentioned species as compared to those on *L. mozardi*. Sensilla chaetica, trichoidea, and basiconica are situated on the apex of the styli and near the base of the styli in *A. obtectus* (Szentesi 1976) and *T. dubius* (Baker and Nebeker 1986) but *L. mozardi* has no sensilla basiconica in this region. The

number of sensilla on the apex of the style of *L. mozdardi* (9–10) is similar to *A. lythri* (8–11) (Phillips 1978) but differs from *A. obtectus* (22–24) and *T. dubius* (23–25) (Szentesi 1976, Baker and Nebeker 1986). The short basiconic sensilla which are situated on the dorsal and ventral surfaces of the ovipositor of *L. mozdardi* are lacking on the ovipositors of the other three beetle species.

The behavioral experiment indicates that the sensilla on the ovipositor of *L. mozdardi* are needed for the deposition of an egg in the plant substrate. Sensilla on the ovipositor of other insects are known to respond to chemicals such as salts, water, and amino acids (Rice 1976, 1977, Chadha and Roome 1980, Hood-Henderson 1982, Liscia et al. 1982). The sensilla trichoidea and basiconica on the ovipositor of *L. mozdardi* may respond to chemicals in the clover plant which are released during the excavation of an oviposition site.

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